Amendments to the Claims

The following list of claims is presented to replace the claims presently on file.

1. A reagent for mass spectrometric analysis of proteins comprising a tag molecule, wherein the tag molecule comprises a reactive site for stably associating with a protein, an isotope label, and a pH sensitive anchoring site for covalently anchoring the tag molecule to a solid phase.
2. The reagent according to claim 1, wherein the anchoring site of the tag molecule forms covalent bonds to a cis hydroxyl pair under selected pH conditions.
3. The reagent according to claim 1, wherein the tag molecule comprises the general formula R-B(OH_2), wherein the R group is a suitable chemical moiety for incorporating the isotope.
4. The reagent according to claim 3, wherein R is selected from the group consisting of an alkyl group, aryl group, heteroaryl group, arylalkyl group, heteroarylalkyl group, and a cyclic molecule.
5. The reagent according to claim 1, wherein the tag molecule is phenyl-$B(OH)_2$ or hexyl-$B(OEthyl)_2$.
6. The reagent according to claim 1, wherein the isotope is selected from the group consisting of a stable isotope hydrogen, a stable isotope of nitrogen, a stable isotope of oxygen, a stable isotope of carbon, a stable isotope of phosphorous and a stable isotope of sulfur.
7. The reagent according to claim 1, wherein the reactive site of the tag molecule is stably associated with a protein.
8. The reagent according to claim 1, wherein the reactive site of the tag molecule is stably associated with a peptide.
9. The reagent according to claim 1, wherein the reactive site group is selected from the group consisting of a chemical moiety which reacts with sulfhydryl groups, a moiety that reacts with amino groups, a moiety that reacts with carboxylate groups, a moiety that reacts with ester groups, a phosphate

reactive group, an aldehyde reactive group, a ketone reactive group and a moiety that reacts with homoserine lactone after fragmentation with CNBr.

10. The reagent according to claim 1, wherein the pH sensitive anchoring group forms a bond with a solid phase under selected pH conditions and wherein the bond is selected from the group consisting of an acyloxyalkyl ether bond, acetal bond, thioacetal bond, aminal bond, imine bond, carbonate bond, and ketal bond.
11. The reagent according to claim 1, 7, or 8, wherein the tag molecule is attached to a solid phase.
12. The reagent according to claim 1, wherein the tag molecule is about 175-300 daltons.
13. The reagent according to claim 3, wherein the isotope is covalently bound to the R group.
14. The reagent according to claim 1, wherein the reactive site forms stable associations with a modified residue of a protein.
15. The reagent according to claim 14, wherein the modified residue is glycosylated, methylated, acylated, phosphorylated, ubiquinated, farnesylated, or ribosylated.
16. A composition comprising a pair of tag molecules according to claim 1, wherein each member of the pair is identical except for the mass of the isotope attached thereto.
17. The composition according to claim 16, wherein one member of the pair comprises a heavy isotope and the other member of the pair comprises the corresponding light form of the isotope.
18. A composition, comprising a reagent for mass spectrometric analysis of proteins comprising a first and second tag molecule, wherein the first tag molecule comprises a reactive site for stably associating with a protein, an isotope label, and a pH sensitive anchoring site for anchoring the tag molecule to a solid phase and the second tag molecule is identical to the first tag molecule but does not comprise an isotope label.

19. A kit comprising at least one reagent according to claim 1 or a composition according to any of claims 16-18, and one or more of a reagent selected from the group consisting of: an activating agent for providing active groups on a protein which bind to the reactive site of the tag molecule; a solid phase; one or more agents for lysing a cell; a pH altering agent; one or more proteases; one or more cell samples or fractions thereof.
20. A kit according to claim 19, wherein the tag molecule further comprises a peptide.
21. A kit comprising a plurality of tagged peptide molecules, each tagged peptide molecule comprising a peptide and a tag molecule stably associated with the protein, the tag molecule further comprising an isotope label, and a pH sensitive anchoring site for anchoring the tag molecule to a solid phase.
22. The kit according to claim 21, wherein the kit comprises pairs of tagged peptides and wherein each member of a pair of tagged peptides comprises an identical peptide and each member of the pair is differentially labeled.
23. The kit according to claim 21, comprising at least one set of tagged peptides; comprising different peptides corresponding to a single protein.
24. The kit according to claim 21, comprising at least one set of tagged peptides comprising peptides corresponding to modified and unmodified forms of a single protein.
25. The kit according to claim 21, comprising at least one set of tagged peptides from a first cell at a first cell state and at least one set of tagged peptides from a second cell at a second cell state.
26. The kit according to claim 25, wherein the first cell is a normally proliferating cell and the second cell is an abnormally proliferating cell.
27. The kit according to claim19, wherein the first and second cells represent different stages of cancer.
28. A method for identifying one or more proteins or protein functions in one or more samples containing mixtures of proteins comprising:

reacting a sample with a first reagent according to claim 1 and a solid phase under conditions suitable to form a solid phase-isotope labeled tag molecule-protein complex;

digesting the complex with one or more proteases, thereby generating solid phase-isotope labeled tag molecule-peptide complexes and untagged peptides;

purifying the solid phase-isotope labeled tag molecule-peptide complexes;

exposing the solid phase-isotope labeled tag molecule-peptide complexes to a pH which disrupts associations between the anchoring site of the tag molecule and the solid phase, thereby releasing a tagged peptide from the solid phase;

determining the mass of the tagged peptide;

correlating the mass to the identity and/or activity of a protein.

29. The method according to claim 28, wherein the mass-to-charge ratio of the tagged peptide is determined.

30. The method according to claim 28, further comprising subjecting a sample comprising one or more tagged peptides to a separation step.

31. The method according to claim 30, wherein the separation step comprises liquid chromatography.

32. The method according to claim 31, comprising subjecting one or more tagged peptides to MS^n analysis.

33. The method according to claim 28, further comprising

reacting a second sample with a second reagent comprising an identical molecular tag as the first reagent but which is differentially labeled.

34. The method according to claim 33, further comprising combining the two samples prior to protease digestion and generating a combined sample comprising at least one pair of tagged peptides, each member of the pair comprising identical peptides but differing in mass.

35. The method according to claim 34, comprising determining the ratio of members of at least one tagged peptide pair in the combined sample.
36. The method according to claim 35, further comprising generating mass spectra comprising at least one signal doublet for each peptide in the sample, the signal doublet comprising a first signal and a second signal shifted a number of known units from the first signal, wherein the known units represent the difference in molecular weight between the two members of a tagged peptide pair.
37. The method according to claim 36, further comprising determining a signal ratio for a given peptide by relating the difference in signal intensity between the first signal and the second signal.
38. The method according to claim 28 or 33, further comprising the step of relating mass spectra data from a tagged peptide to an amino acid sequence.
39. The method according to claim 28, wherein the steps of the method are repeated, either sequentially or simultaneously, until substantially all of the proteins in a sample are detected and/or identified.
40. The method according to claim 33, wherein the relative amounts of members of a tagged peptide pair in the two samples are determined and correlated with the abundance the protein corresponding to the peptide in the sample.
41. The method according to claim 40, further comprising correlating the relative abundance of the protein with the state of the cells.
42. The method according to claim 41, wherein correlating is used to diagnose a pathological condition in a patient from whom one of the cell samples was obtained.
43. The method according to claim 28, comprising determining the quantity of a protein corresponding to the peptide in the sample.
44. The method according to claim 28 or 33, comprising determining the site of a modification of a protein in one or more samples, by reacting sample proteins with a tag molecule comprising a reactive site which reacts with a modified residue on the protein.

45. The method according to claim 42, further comprising determining the amount of modified protein in the sample.